HexaLabel™ DNA Labeling Kit

#K0611
Lot: ___ Exp.: ___
Quantity: for 10 reactions

#K0612
Lot: ___ Exp.: ___
Quantity: for 30 reactions

WARNING!
Store at -20°C.
Repeated freeze-thawing diminishes the quality.

DESCRIPTION

Labeling of DNA by random oligonucleotide-primed synthesis is based on the investigations of A.Feinberg and B.Vogelstein [1, 2] and is a good alternative to nick translation for producing uniformly radioactive DNA of high specific activity. The method relies on priming of the polymerase reaction on the template DNA with random hexanucleotide primers. The complementary strand is synthesized from the 3'-end of the primer with the help of DNA Polymerase I, Exonuclease Minus (Klenow Fragment, exo-) in the presence of labeled deoxyribonucleoside triphosphates. Using Klenow Fragment, exo- the reaction time can be prolonged without the fear of labeled probe degradation.

The **HexaLabel™ DNA Labeling Kit** offered by Fermentas is an efficient and convenient means for preparation of highly labeled DNA (>10^9dpm/µg) for use diverse procedures in molecular biology, such as various types of hybridization analyses [3-6]. Either dATP or dCTP could be used with the kit as a radioactive precursor.

Now, non-radioactive labeling Mix (-DIG-dUTP) is also included in the kit as an alternative labeling of DNA (DIG-dUTP is not included).

COMPONENTS OF THE KIT

1. **Klenow Fragment, exo-, 5u*/µl:**
   15µl (30µl) of the enzyme solution in buffer containing 50% glycerol.

2. **Hexanucleotide in 5X Reaction Buffer:**
   100µl (300µl) of 0.25M Tris-HCl buffer (pH 8.0 at 20°C) containing 25mM $MgCl_2$, 5mM dithiotreitol and Random (hexamer) primer (7.5o.u./ml).
3. **Mix A (minus dATP):**
   30µl (90µl) of 0.33mM dGTP, 0.33mM dTTP, 0.33mM dCTP aqueous solution.
4. **Mix C (minus dCTP):**
   30µl (90µl) of 0.33mM dGTP, 0.33mM dATP, 0.33mM dTTP aqueous solution.
5. **dNTP Mix:**
   40µl (120µl) of 0.25mM dGTP, 0.25mM dATP, 0.25mM dTTP, 0.25mM dCTP aqueous solution.
6. **Control Template:**
   for 2 (5) control labeling reactions, 20µl (50µl) of lambda phage DNA/*Hin*dIII fragments (10µg/ml).
7. **Non-radioactive Labeling Mix (minus DIG-dUTP):**
   50µl (150µl) of 1mM dGTP, 1mM dATP, 1mM dCTP, 0.65mM dTTP aqueous solution.
8. **Deionized Water:**
   1.5ml of water deionized on a Milli-Q® system.

** One unit of Klenow fragment, exo- catalyzes the incorporation of 10nmoles of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 37°C, using poly(dA-dT)·poly(dA-dT) as a template · primer.*

*** If you use ethanol solution of labeled [alpha-^{32}P]-dATP or [alpha-^{32}P]-dCTP, the needed quantity of these nucleotides dry under vacuum and redissolve in 6µl of deionized water.*

EXPERIMENTAL PROTOCOL

Radioactive DNA Labeling

1. Add the following components into 1.5ml microcentrifuge tube:

DNA template (100ng)	10µl
hexanucleotide in 5X reaction buffer	10µl
deionized water	to 40µl

   Vortex the tube and spin down in a microcentrifuge for 3-5sec.
   Incubate the tube in a boiling water bath for 5-10min and cool it on ice. Spin down quickly.

2. Based on your choice of labeled triphosphate (dATP or dCTP) use Mix A or Mix C, respectively.

3. Add the following components in the same tube:

Mix A (or Mix C)	3µl
[alpha-^{32}P]-dATP (or [alpha-^{32}P]-dCTP)** (1.85MBq = 50µCi)	6µl
Klenow Fragment, exo- (5u)	1µl

   Shake the tube and spin down in a microcentrifuge for 3-5sec.
   Incubate for 10min at 37°C.

4. Add 4µl of dNTP Mix and incubate for 5min at 37°C.

5. Stop the reaction by the addition of 1µl 0.5M EDTA, pH 8.0.

6. The labeled DNA is used directly for hybridization or stored at -20°C. Removal of the unincorporated label is not necessary for most applications, since the levels of its utilization are usually high. If required, the unincorporated dNTP can be removed by chromatography on Sephadex® G-50 or by selective precipitation of DNA with ethanol in the presence of ammonium acetate [7].

Determination of the Percentage of Label Incorporation

The percentage of incorporation is determined by DE-81 filter-binding assay.

1. Dilute 1µl of the reaction mix 1:100 with 0.2M EDTA or water. Spot 5µl of it on each of the two Whatman® DE-81 filters (1.5 x 1.5cm).

2. Dry the filters under a heat lamp. Keep one filter aside and use it directly for the determination of total dpm in the sample.

3. Wash the other filter 3 times for 5min in 10ml 7.5% (w/v) $Na_2HPO_4 \cdot 12H_2O$ for the removal of the unincorporated dNTPs, then once in water and in acetone.

4. Dry the washed filter under a heat lamp.

5. Transfer and count the filters in an appropriate radioactivity counter.

6. The percentage of label incorporation into DNA is defined as below:

$$\frac{\text{incorporated radioactivity (washed filter)}}{\text{total radioactivity (unwashed filter)}} \times 100\%$$

7. Calculate the specific activity of the probe:
for the calculation of the specific activity of the labeled DNA probe, it is first necessary to evaluate the theoretical amount of DNA generated in the reaction assuming that 100% incorporation of radioactivity occurred:

$$\frac{\mu\text{Ci dNTP added} \times 4 \times 330\text{ng/nmole}}{\text{specific activity dNTP (Ci/mmole= µCi/nmole)}} = \text{ng theoret. yield}$$

330ng/nmole = average molecular weight of a nucleotide.
Further, calculate the percentage incorporation according to DE-81 filter-binding assay results:

$$\frac{\text{dpm incorporated}}{\text{total dpm}} \times 100\% = \%\text{ incorporation}$$

Determine the amount of DNA synthesized:

$$\%\text{ incorporation} \times 0.01 \times \text{theoret. yield} = \text{ng DNA synthesized}$$

Calculate the specific activity of the product:

$$\frac{\text{total dpm incorp.(dpm incorp. x 20 x 50)}}{\text{(ng DNA synth. + ng input DNA) x 0.001}\mu\text{g/ng}} = \text{dpm}/\mu\text{g}$$

Note

The factors 20 and 50 are derived while using 5µl of 1:100 dilution for DE-81 filter-binding assay and converting this back to 50µl total reaction volume.

Example. *If you use 50µCi (1.85 MBq) [alpha-^{32}P]-dCTP (3000Ci/mmole) in a standard reaction, the calculation is as follows:*

$$\frac{50\mu\text{Ci x 4 x 330 ng/nmole}}{3000\mu\text{Ci/nmole}} = 22\text{ng theoretical yield}$$

Assuming that 14.91 x 10^4dpm remained on the washed DE-81 filter and the unwashed filter retained 18.12 x10^4dpm, we recur:

$$\frac{14.91 \times 10^4}{18.12 \times 10^4} \times 100\% = 82.3\%\text{ incorporation}$$

$$0.823 \times 22\text{ng} = 18.1\text{ng DNA synthesized}$$

Thus, the specific activity is:

$$\frac{14.91 \times 10^4\text{dpm x 20 x 50}}{\text{(18.1ng + 100ng) x 0.001}\mu\text{g/ng}} = 1.26 \times 10^9\text{dpm}/\mu\text{g}$$

Labeling of DNA with DIG-dUTP

1. Add the following components into 1.5ml microcentrifuge tube:

DNA template (100ng-1µg)	10µl
random primer in 5X reaction buffer	10µl
deionized water	to 42µl

Vortex the tube and spin down in a microcentrifuge for 3-5sec.
Incubate the tube in a boiling water bath for 5-10min and cool it on ice. Spin down quickly.

2. Add the following components in the same tube:

Non-radioactive Labeling Mix (-DIG-dUTP)	3µl
1mM DIG-dUTP (not included in the kit)	1.75µl
Klenow Fragment, exo- (5u)	1µl

Shake the tube and spin down in a microcentrifuge for 3-5sec.
Incubate for 1 hour at 37°C. Prolonged incubation at 37°C up to 20 hours increases the yield of labeled DNA.

3. Stop the reaction by the addition of 1µl 0.5M EDTA, pH 8.0.

4. The labeled DNA is used directly for hybridization or stored at -20°C. Removal of the unincorporated label is not necessary for most applications. If required, the unincorporated dNTP can be removed by chromatography on Sephadex® G-50 or by selective precipitation of DNA with ethanol in the presence of ammonium acetate [7].

Note

1. Using 100ng template DNA can be synthesized about 50-75ng of DIG-labeled DNA after 1 hour incubation and about 240-270ng after 20 hours incubation at 37°C.
2. Other non-radioactive labels (biotin-, fluorescein- dUTP) can be used.

QUALITY CONTROL

All components of the Kit are tested in a labeling reaction of lambda DNA/*Hin*dIII fragments, obtained specific activity $1.4x10^9$dpm/µg DNA.

References

1. Feinberg, A.P., Vogelstein, B., Biochem. 132, 6-13, 1983.
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4. Meinkoth, M., Wahl, G., Anal. Biochem. 158, 267-284, 1984.
5. Grunstein, M. Hogness, D.S., Proc. Natl. Acad. Sci. USA 72, 3961-3965, 1975.
6. Southern, E.M., J. Mol. Biol., 98, 503-517, 1975.
7. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual; Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989.

Product Use Limitation

MSDS